# Development of RT-PCR Primers for Examination of Cell Proliferation and Neural Gene Expression in Mosquitoes

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## Abstract

The Zika virus (ZIKV) is a flavivirus carried by mosquitoes in the *Aedes* genus. The recent epidemic of Zika infection carried by *Aedes aegypti* in Brazil became a global matter when a significantly increased number of infants were found to have microcephaly and other birth defects in areas with ZIKV.[1] In Texas, there have been 363 cases of Zika as of March 21, 2018, 9 of which were acquired by local mosquito-borne transmission.[2] There have been recent studies that show that ZIKV infection has associations with reduced cell proliferation in mammalian neural tissue, and thus hypoplasia, which is found in microcephaly.[1, 3] It is still not clear if vectors are subject to similar influence. My goal is to explore whether ZIKV changes gene expression in brain tissue of *Aedes aegypti* mosquitoes. Because there has been little research on cell proliferation and neural development genes in mosquitoes, in this project, we seek to profile some of those genes. Therefore, I found studies that looked at those genes in *Drosophila melanogaster* and performed *in silico* searches for their homologs in *Aedes aegypti* mosquitoes. 18 total primers were designed for 5 presumed *Aedes aegypti* homologs to allow for RT-PCR gene expression quantitation in mosquito brains. By utilizing various combinations of primers, we will be able to test for 32 amplicons. Testing of the primer efficacy and of gene expression is ongoing.

## Background

- The Zika virus has been found in all of the states of the United States. In Texas, there have been 363 cases of Zika as of March 21, 2018, 9 of which were acquired by local mosquito-borne transmission.[2]
- ZIKV has been found to be associated with microcephaly in infants. Microcephaly is caused by reduced proliferation of cortical progenitor cells in the brain, causing the head circumference to be reduced at birth.[1]
- Microcephaly has also been associated with impairment in cognitive development, motor functions, and other brain abnormalities.[4] It can infect human progenitor cells and reduce their growth, production, or proliferation.[1]
- These studies show that ZIKV infection has associations with reduced cell proliferation and thus hypoplasia which is found in microcephaly.

## Methods

A literature review was first conducted to determine prominent genes involved in microcephaly, cell proliferation, or neural development and to find published primers for those genes for *Drosophila melanogaster* and *Aedes aegypti*. Seven published primer sets for 4 genes (*Rpl32, Gapdh, Actin, Mcph1*) involved in microcephaly, cell proliferation, or neural development were found in previous studies. The rest of the genes (*Asp, Mcph1, Cdk4*) without published primers were newly designed (Figure 1).

The mRNAs for each gene from *D. melanogaster* and *A. aegypti* were aligned using the National Center for Biotechnology Information (NCBI) website.[5]

Common primers shared by two species were attempted to be designed based on the conserved regions using PrimerIdent.[6]

Separate sets of primers were designed using NCBI's PrimerBlast as shown in the UCSC Genome Browser (Figure 1).[7]

Only primers were selected if there were at least one intron flanked by the pair of primers.

Four primers were selected for each gene if possible for increased different combinations with higher success rates.

To validate the specificity and efficacy of designed primer, wild-type *D. melanogaster* flies were disrupted using a mortar and pestle and homogenized with QIAshredder.

RNA was extracted using the QIAGEN RNeasy Mini Kit. Primer concentrations used in RT-PCR were 10uM.

RT-PCR experiments were run using the QIAGEN One-Step RT-PCR Kit. There were 40 cycles, with denaturation at 94°C, annealing at 60°C, and extension at 72°C.

RT-PCR products were fractioned and visualized using gel electrophoresis with Invitrogen E-Gels.

## Preliminary Results

Our results (Figure 3) indicate that the D. melanogaster RNA extraction and RT-PCR were successful for the *Rpl32*, *Gapdh*, and *Actin* genes and that the primers are working. The gel shows no bands in the negative control (NTC, or No Template Control), indicating that there was no contamination. The brightest bands are the expected product. The lighter bands may be non-specific amplification or may represent low level DNA amplification. The bright band should be RNA amplification, rather than DNA because they are of the correct size, and the spin columns were resistant to DNA elution.

| Gene and Species | Expected Amplicon Size | Sequence | Previously Published | Reference |
|---|---|---|---|---|
| Rpl32 (D. melanogaster) | 132 bp | Forward 5'-TGTCCTTCCAGCTTCAAGATGACCATC-3'<br>Reverse 5'-CTTGGGCTTGCGCCATTTGTG-3' | Yes | Brunk et al. (2007) |
| Rpl32 (D. melanogaster) | 133 bp | Forward 5'-AAGCGGCGACGCACTCTGTT-3'<br>Reverse 5'-GCCCAGCATACAGGCCCAAG-3' | Yes | Ling et al. (2011) |
| Gapdh (D. melanogaster) | 143 bp | Forward 5'-CCACTGCCGAGGAGGTCAACTAC-3'<br>Reverse 5'-ATGCTCAGGGTGATTGCGTATGC-3' | Yes | Ling et al. (2009) |
| Actin (D. melanogaster and A. aegypti) | 683 bp | Forward 5'-ATGGTCGGYATGGGNCAGAAGNGACTC-3'<br>Reverse 5'-TCGCACTTCATNGATSGAGTTGTA-3' | Yes | Staley et al. (2010) |
| Asp (D. melanogaster) | 174 bp | Forward 5'-CCATCGCGCCTAAAGCAAAA-3'<br>Reverse 5'-TACCTTGACCTCGATGTCGTC-3' | No | N/A |
| Asp (D. melanogaster) | 182 bp | Forward 5'-GACGACATCGAGGTCAAGGTA-3'<br>Reverse 5'-TCCTTTCGGAAGTTGCGGTT-3' | No | N/A |
| Mcph1 (D. melanogaster) | 653 bp | Forward 5'-GCAATAAGGAGCGCCAGAGTG-3'<br>Reverse 5'-ACGCCCTCAGAGCGATTGTC-3' | Yes | Brunk et al. (2007) |
| Mcph1 (D. melanogaster) | 187 bp | Forward 5'-CAATCCCACGTGGAAGACGA-3'<br>Reverse 5'-GGCCGCCTGGTCATCTG-3' | No | N/A |
| Cdk4 (D. melanogaster) | 117 bp | Forward 5'-CTCAACTCTTACGAATATTGCTGGT=3'<br>Reverse 5'-CGCTTCAGCTGGCGTACATA-3' | No | N/A |
| Cdk4 (D. melanogaster) | 166 bp | Forward 5'-CTCTTACGAATATTGCTGGTGC-3'<br>Reverse 5'-TGGTAGTTGAACGGATCGCC-3' | No | N/A |
| Rpl32 (A. aegypti) | 200 bp | Forward 5'-CAGTCCGATCGCTATGACAA-3'<br>Reverse 5'-ATCATCAGCACCTCCAGCTC-3' | Yes | Dzaki et al. (2017) |
| Gapdh (A. aegypti) | 194 bp | Forward 5'-ACAGACGCTAGTTATCAACGTA-3'<br>Reverse 5'-ACCGTGGGTCGAATCGTA-3' | Yes | Dzaki et al. (2017) |
| Asp (A. aegypti) | 137 bp | Forward 5'-CTCGATGCAGAGGAGTTCGT-3'<br>Reverse 5'-ATTTGGGAACCTGAAAGCACG-3' | No | N/A |
| Asp (A. aegypti) | 198 bp | Forward 5'-CGTGCTTTCAGGTTCCCAAAT-3'<br>Reverse 5'-TAGTGGTCAGGAAGGGGTCG-3' | No | N/A |
| Mcph1 (A. aegypti) | 102 bp | Forward 5'-TTCGCTCCGGAACAGACAAT-3'<br>Reverse 5'-TAACGTGCGTTGTGTCTTTGT-3' | No | N/A |
| Mcph1 (A. aegypti) | 117 bp | Forward 5'-GTACAAAGACACAACGCACG-3'<br>Reverse 5'-TGCCGCTTACATGCTTCGAT-3' | No | N/A |
| Cdk4 (A. aegypti) | 108 bp | Forward 5'-CAGGAGCATATGGCACCGTC-3'<br>Reverse 5'-ACATTGGCACTCCATCCTCG-3' | No | N/A |
| Cdk4 (A. aegypti) | 159 bp | Forward 5'-AGGCACGTGACCTTAGCAAT-3'<br>Reverse 5'-CGTCCAGCAATTTGACAACA-3' | No | N/A |

**Figure 1.** Table displaying the gene names, species, amplicon sizes, primer sequences, and references.

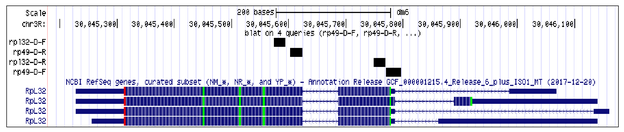


**Figure 2.** Schematic illustration of the *Rpl32* primers (black bars) aligned to the *Rpl32* gene variants with exons (blue bars) and introns (thin blue lines)

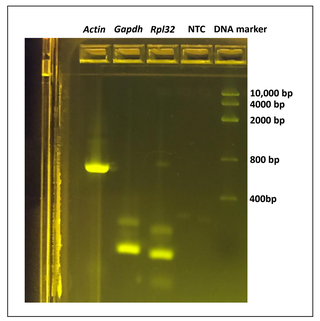


**Figure 3.** Visualization of RT-PCR products for *Actin*, *Gapdh*, and *Rpl32* using gel electrophoresis for *D. melanogaster*.

## Future Direction

The preliminary results indicated that RNA extraction and RT-PCR were successful, so for future direction, I will evaluate the rest of the primers for *D. melanogaster* that serves as a positive control to determine the best primer set for each gene. Once I catch mosquitoes, I will test the *A. aegypti* primers and determine the best primer set for each gene on mRNA from mosquitoes. I will then test for the presence of ZIKV in the mosquitoes. I will run RT-PCR reactions to check any differences in gene expression in the brain tissue of ZIKV-positive mosquitoes and ZIKV-negative mosquitoes identified using ZIKV specific primers. Reduced transcripts of these 5 neural and proliferative genes would be anticipated in Zika-positive mosquitoes if ZIKV inflicts damage and apoptosis in stem cells in the brain of vectors. I will then compare their expression to published data of human expression of those genes.

## References

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